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INTOLERANCE TO MILK PROTEIN

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Intolerance to milk protein

Six cases of gastrointestinal milk allergy were investigated. Vomiting with or without shocklike symptoms, failure to thrive, and diarrhea were the presenting symptoms. In 5 of the 6 patients, beta lactoglobulin was toxic; in 4 of these it was the only toxic protein. Hemagglutinating and precipitating antibodies helped in establishing the diagnosis in 3 cases. Steatorrhea was found in none of the 4 patients in whom it was sought. Intestinal biopsy was abnormal in 1 of 2 cases.

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ALLERGY to cow's milk protein has become more clearly defined during the last few years. Among the clinical features are gastrointestinal manifestations such as diarrhea and vomiting; these are often associated with shock. Six infants with one or more of these symptoms are described. An effort was made to identify the milk proteins responsible for producing the symptoms, to assess levels of immunoglobulins and of milk-protein antibodies in the serum, and to measure intestinal absorption by various parameters.

METHODS

Isolated milk proteins were administered in 50 to 100 ml. of water sweetened with sucrose. They were obtained from the following sources: casein from British drug houses; crystallized bovine serum albumin and bovine gamma globulin from Armour Pharmaceutical Co.; beta lactoglobulin, 3 times crystallized, from Nutritional Biochemicals Corp.; alpha lactalbumin, purified, from Pentex, Inc. The cow's milk used was reconstituted from Nursia whole-milk powder (United Dairymen N.V., Holland). By testing the beta lactoglobulin with anti–cow's milk serum it was found that other proteins, especially alpha lactalbumin, were present in trace amounts. In order to ascertain the antigenicity of the types of milk used in our challenges, we immunized 2 rabbits with each preparation. Sera from these immunized rabbits were tested for hemagglutinating and precipitating antibodies against the isolated milk proteins used in the challenges (apart from casein). The modified powdered milk preparations used in the challenges were reconstituted with lukewarm water to minimize the effects of heat on the antigenicity of the milk.

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The passive hemagglutination technique of Boyden[1] was employed with some modification.[2] The radioimmunodiffusion technique was based on the radioimmunoelectrophoresis method of Yagi and associates.[3] Goat antisera against human immunoglobulins IgA and IgM (Hyland Labs., Los Angeles, Calif.) were tested against human serum by immunoelectrophoresis and found to be monospecific. Goat antihuman IgG was prepared in our laboratory; this was found to be monospecific. Four ml. of 1.5 per cent Noble Difco agar in Veronal buffer pH 8.6 was poured on glass slides. Fifteen wells were made and about 15 μl of serum and antiserum placed in alternate wells. On each slide only one type of antiserum was used. After the development of the precipitate the slides were washed for 48 hours with 4 changes of saline in order to remove the unprecipitated proteins. Thereafter 15 μl of milk protein iodinated by Yagi's modification of the chloramine T method were applied to each antiserum well containing about 3 μg of protein and 10 μc of I^{125}. Thus the specific activity was 3.3 μc per 1 μg protein. After 24 hours the unbound protein was washed with 4 changes of 0.1 per cent potassium iodide in saline for 2 to 3 days. The slides were dried and stained with amido black. The stained slide was wrapped in Saran Wrap (Dow Chemical Co., Midland, Mich.) and attached to x-ray film (Kodak, type KK). After incubation in the dark for 10 to 14 days, the film was developed and the antibody activity in the tested sera determined by the density of the lines produced on the film due to irradiation by trapped iodinated antigen.

The gel-diffusion test was carried out on agar slides. Six wells were made surrounding a central one. The distance between the wells was 8 mm. Serum samples were applied to the central well; whole milk or isolated milk proteins in concentrations of 2.5, 5, and 10 mg. per milliliter were put into the peripheral wells. The reactions were read after incubation at room temperature for 24 and 48 hours. A standard rabbit anti–whole milk serum was included on each slide as a positive control.

D-xylose was estimated by the method of Roe and Rice.[4] Fat in stools was measured by the method of Jover and Gordon[5] in Patients 1, 5, and 6, and according to van de Kamer[6] in Patient 2. Immunoglobulins were determined by the radial immunodiffusion method of Mancini and associates.[7] Blood glucose was estimated by the glucose oxidase method.[8] Intestinal biopsies were taken from the fourth part of the duodenum with an infant-size Crosby-Kugler capsule.

CASE REPORTS

Only the main clinical features are described. Both sexes were equally involved, and the infants belonged to Oriental and Ashkenasic Jewish as well as to the Arab communities. A positive family history suggestive of temporary milk allergy in a parent or sibling was obtained in Patient 4 only; there was no family history of other allergy

Table I. Tests of intestinal function

Test	*Patient No.*			
	1	*2*	*5*	*6*
Glucose tolerance	Normal	—	—	Low
Sucrose tolerance	Low	Normal	—	Normal
Lactose tolerance	Normal	Normal	—	Low
Fat balance (on beta lactoglobulin)	Normal	Normal	—	—
Fat balance (on soy formula only)	Normal	Normal	Normal	Normal*

Sugar tolerance tests were performed by administering 2 Gm per kilogram of the sugar concerned, and taking capillary samples of blood every 20 minutes. A rise of over 30 mg. percent for glucose or sucrose, and over 15 mg. percent for lactose, was considered normal.

*Immediately after discontinuing cow's milk.

in any of the patients. In 4 of 6 infants, isolated feedings of cow's milk during the first days of life were well tolerated; in the other 2 information on this point was incomplete. Allergy to cow's milk was manifest in 4 infants during the first month of life and in the other 2 at 5 and 10 weeks, respectively. In 3 infants vomiting and pallor were produced by each of 3 separate trials with cow's milk. Two of these infants were weaned onto soybean milk immediately. The third infant did not vomit during a fourth challenge with cow's milk, but when the mother continued to give cow's milk he developed diarrhea with mucus and ultimately had to be fed soybean milk as well. Two infants had persistent severe diarrhea and required repeated intravenous infusions for one month until the cause of their diarrhea was recognized and cow's milk replaced by a soybean formula. The diarrhea ceased gradually during the course of 3 weeks. The sixth infant presented with vomiting, abdominal distension, and failure to gain. Extensive investigations for partial intestinal obstruction were negative. Change to soybean formula resulted in sudden gain of weight and cessation of vomiting. Patients 1 and 3 have lost their sensitivity, one at 6 months and one at one year; the others are still under observation.

ROUTINE INVESTIGATIONS

Patients 1, 2, 5, and 6 were hospitalized for study. Urinalysis, hemoglobin, white blood cell count, serum electrolytes and proteins, blood urea nitrogen and fasting blood glucose values were normal. Glucose and sucrose tolerance tests showed slight deviations in some of the tests. The lactose tolerance test was low in Patient 6, but primary lactase deficiency could be excluded because the infant thrived and was without symptoms during the first 5 weeks of life despite receiving human and cow's milk. The results of fat balance studies in 2 cases while on beta lactoglobulin, and in a third case immediately after prolonged administration of cow's milk, were normal (Table I). Intestinal biopsy was performed on Patient 1 after she had been on soybean formula for 6 months. Under the dissecting microscope, leaflike villi were seen. The microscopic appearance was normal. In Patient 2, who had been on soybean formula for 2 months, the villi were stunted. Inflammatory cells were increased in the lamina propria and invaded the

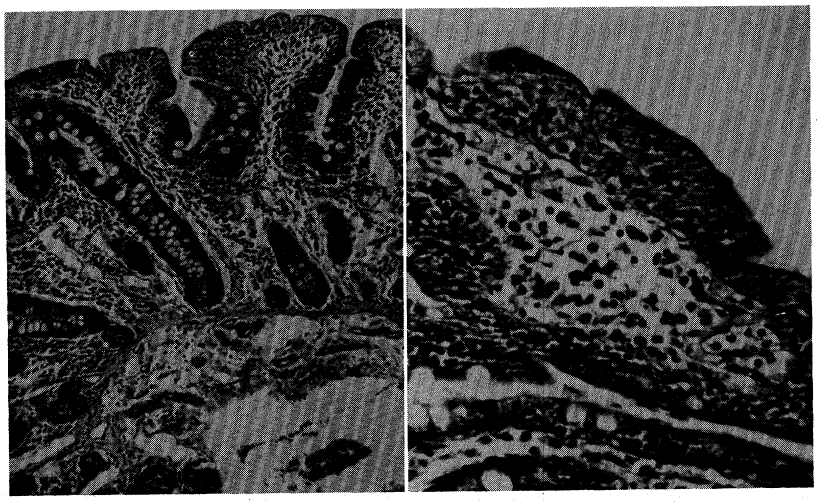

Fig. 1. Duodenal biopsy in Patient 2. *Left,* The villi are short and broad, and there is an increase of cellularity. (× 180.) *Right,* Inflammatory cells can be seen invading the epithelium. (×500.)

Table II. Challenges

Substance	Quantity*	Patient No. 1	2	3	4	5	6
Cow's milk	5 ml.	D, P, WT	—	V, P, WT	—	—	—
Cow's milk	50 ml.	V, D, P, WT	V, D, WT, CY, WHE, FE	—	V, D	—	V, WT
Cow's milk	100 ml.	—	—	V, D, P, WT	—	V, WT	—
Goat's milk	50 ml.	—	V, D, WT, CY, WHE, FE	—	—	0	0
Beta lactoglobulin	100 mg. (30)	V, D, P, WT	V, D, WT, CY, WHE, FE	V, D, P	D	V, WT	0
Bovine serum albumin	500 mg. (1,660)	0	0	0	0	D, FE	V, D, WT†
Bovine gamma globulin	1,000 mg. (600)	0	0	0	0	V, WT	0
Alpha lactalbumin	1,000 mg. (700)	0	0	0	0	V, D, WT	0
Casein	2,000 mg. (80)	0	0	0	0	0	0
Allergilac	100 ml.	V, D, P, WT	—	—	D	—	0
Eledon	100 ml.	V, D, P, WT	—	—	—	—	V
Similac	100 ml.	V, D, P, WT	—	—	—	V, D, WT	D
Nutramigen	100 ml.	0	—	—	—	—	—

Abbreviations: D = diarrhea, V = vomiting, P = pallor, WT = loss of weight, CY = cyanosis, WHE = wheezing, FE = fever, 0 = no reaction, — = not given.

*Figures in parentheses denote the amount of milk in milliliters which would contain the respective protein.

†Reacted to 80 mg. BSA.

epithelium (Fig. 1). The epithelial cells were normal.

In some cases as little as 5 ml. of cow's milk produced symptoms (Table II). Goat's milk was tolerated in 2 of 3 patients. Of the individual milk proteins, beta lactoglobulin was toxic in 5 of 6 infants; in 4 of these it was the only toxic protein. In Patient 1, a challenge of beta lactoglobulin which had been boiled for 70 minutes caused a severe reaction. Patient 6 was sensitive to bovine serum albumin only. No infant was sensitive to casein. Allergilac was not tolerated by one infant sensitive to beta lactoglobulin, but was tolerated by the infant sensitive to bovine serum albumin. Eledon and Similac were not tolerated by either. Nutramigen was tolerated by the infant who had severe reactions to 5 ml. of cow's milk and to beta lactoglobulin which had been boiled for 70 minutes. An enema of 50 ml. of cow's milk in Patient 1 also produced diarrhea and loss of weight; the symptoms in this patient were usually pallor, vomiting, diarrhea, and loss of weight. Patient 2 usually became cyanosed and developed wheezing and a fever. It was noticed that if challenges were repeated on successive days the severity of the symptoms lessened.

The levels of the immunoglobulins are recorded in Table III. IgA was elevated in Patient 1 and IgG reduced in Patient 3. The

levels of serum immunoglobulins were otherwise unremarkable.

MILK ANTIBODIES

The distribution of milk antibodies among the various immunoglobulins is probably high in Patients 2 and 6 (Table IV). In 40 control subjects the mean values at 4 months were IgG 2.8, IgA 1.2, and IgM 0.2. At 9 months the values were IgG 1.6, IgA 0.8, and IgM 0.1. Hemagglutinating antibodies to whole milk or to the purified proteins tested were high in Patients 1, 2, and 6. In our laboratory, among 800 control subjects, hemagglutinating antibodies to whole milk in a dilution of 1:640 were always significant of specific pathology. Similar antibodies to purified milk proteins were tested in 10 control subjects only. The highest level was 1:320. The infants with increased hemagglutinating antibodies also had positive precipitation reactions in gel (Fig. 2). Fifty controls had negative reactions.

Table III. Immunoglobulins

Patient No.	*Age (mo.)*	*Immunoglobulins (mg. %)*		
		IgG	*IgM*	*IgA*
1	7	521	71	131*
2	11	Normal	Normal	Normal
3	3	195†	52	39
4	8	322	54	22
5	3	409	67	22
6	4	—	—	—

*Slightly elevated.

†Reduced.

ANTIGENICITY OF MILK PREPARATIONS

The antigenicity of the milk preparations used can be seen in Table V. Both by gel precipitation and by hemagglutination, whole milk was found to be the most antigenic. Reconstituted whole milk (Nursia) and reconstituted modified milks (Similac and Allergilac) had a similar degree of allergenicity. Nutramigen, although it contains hydrolyzed protein, was still shown to produce antibodies against all of the isolated milk proteins which were tested. (For technical reasons hemagglutinating antibodies to casein could not be determined.)

DISCUSSION

In an extensive cooperative study of milk sensitivity, Goldman and associates[9] found vomiting and/or diarrhea to be the presenting symptom in 53 per cent of patients with milk allergy, and anaphylaxis in 4 per cent. They investigated 45 proved milk-sensitive children in an effort to establish which of 4 milk proteins was responsible for symptoms. In infants suffering from anaphylaxis, diarrhea, or vomiting, beta lactoglobulin was not more frequently toxic than the other milk proteins tested—casein, alpha lactalbumin, and bovine serum albumin. On the other

Table IV. Milk antibodies

Patient No.	*Age (mo.)*	*Radioimmunodiffusion* Antigen: Cow's milk*			*Hemagglutination†*						*Precipitins*					
		IgG	*IgA*	*IgM*	*Cow's milk*	*BSA*	*BLG*	*ALA*	*Casein*	*BGG*	*Cow's milk*	*BSA*	*BLG*	*ALA*	*Casein*	*BGG*
1	4	3	1	0	4	6	6	6	–	5	++	±	++	++	0	+
2	10	5	3	2	6	5	6	5	6	6	+	0	+	+	0	±
3	4	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
4	6	1	1	0	1	4	3	2	3	2	0	0	±	0	0	0
5	3	–	–	–	2	3	3	3	3	3	0	0	0	0	0	0
6	4	6	5	2	7	9	7	9	5	8	+++	+	++	++++	0	0

Abbreviations: BSA = bovine serum albumin, BLG = beta lactoglobulin, ALA = alpha lactalbumin, BGG = bovine gamma globulin.

*Numbers refer to an arbitrary gradation describing density of line.

†Numbers refer to last test tube showing positive results. Successive test tubes contained twofold serial dilutions, Tube No. being 1:20.

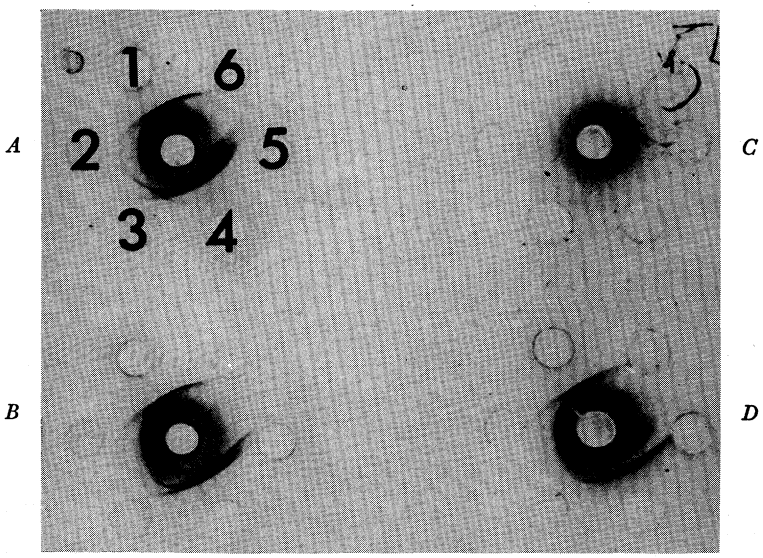

Fig. 2. Precipitation in gel between the serum of an infant with milk sensitivity (Patient 6) and milk components (*A, B, C*) and soybean preparation (*D*). The infant's serum is in the central wells; the antigens are in the peripheral ones. Milk components: 1, whole milk; 2, BSA; 3, BLG; 4, ALA; 5, casein; 6, BGG. These antigens were tested in the following concentrations: A, 10 mg. per milliliter; B, 5 mg. per milliliter; C, 2.5 mg. per milliliter. Soybean preparation was tested in double dilution, wth 4 mg. per milliliter in peripheral well No. 1.

hand, 3 of the 4 patients described by Visakorpi and Immonen[10] were sensitive to beta lactoglobulin, as well as to some other milk proteins. In our series, 5 of the 6 infants were sensitive to beta lactoglobulin, 4 of them to this protein alone. Although the beta lactoglobulin we used was contaminated by alpha lactalbumin, the toxic effects can be ascribed to the former, because in 5 of the 6 patients alpha lactalbumin alone had no deleterious effects. It appears that beta lactoglobulin is among the more allergenic of the milk proteins in human beings.

Ratner and associates[11] found beta lactoglobulin to be the most antigenic of the milk proteins, while Cole and Dees,[12] using a similar technique, ascribed this property to alpha lactalbumin. Normal milk contains approximately 2.5 Gm. per cent of casein, 300 mg. per cent of beta lactoglobulin, 70 mg. per cent of alpha lactalbumin, 30 mg. per cent of bovine serum albumin, and 60 mg. per cent of immunoglobulin.[13] It is customary[9] to challenge with milk proteins in amounts which are contained in 100 ml. of milk. In view of the severe symptoms produced by beta lactoglobulin in some cases, we challenged with very much smaller quantities of this protein. On the other hand, the doses of other proteins used in the challenges were comparatively large.

Three genetic variants of beta lactoglobulin in cow's milk have been identified by starch-gel electrophoresis and designated as A, B, and C. A and B phenotypes are by far the most common, most cow's milk being either A/A, A/B, or B/B. All three genetic variants of bovine beta lactoglobulin are immunologically identical. Furthermore, the beta lactoglobulins of sheep's and cow's milk are also identical immunologically. Human milk contains no beta lactoglobulin.[14]

The fact that Patient 2 reacted violently to goat's milk suggests that cow and goat beta lactoglobulins are also immunologically identical. This has been shown to be true in vitro.[15] On the other hand, Hill[16] mentions that all of 15 infants who had an anaphylactoid reaction to cow's milk tolerated goat's milk. This was true in one of our patients

Table V. Reactions of rabbit antisera to 5 milk products with milk antigens

Serologic technique	*Rabbit No.*	*Immunizing preparation*	*Whole milk*	*Milk antigens*				
				ALA	*BLG*	*BSA*	*BGG*	*Casein*
Gel precipitation*	1	Whole milk	+++	+++	++	(+)	++	0
	2	Whole milk	+++	++	+++	(+)	++	0
	3	Nursia	0	0	0	0	0	0
	4	Similac	+	0	(+)	0	0	+
	5	Similac	0	0	0	0	0	0
	6	Allergilac	0	0	0	0	0	0
	7	Allergilac	+	++	(+)	0	+	0
	8	Nutramigen	0	0	0	0	0	0
Hemagglutination†	1	Whole milk	8	12	12	11	13	-
	2	Whole milk	6	11	12	9	13	-
	3	Nursia	5	8	9	8	7	-
	4	Similac	6	8	8	5	9	-
	5	Similac	4	6	4	0	0	-
	6	Allergilac	5	9	9	7	7	-
	7	Allergilac	6	12	10	10	9	-
	8	Nutramigen	1	6	4	3	5	-

For Abbreviations, see legend to Table IV.

*Precipitation results are expressed by an arbitrary scale of 0 to ++++ for the intensity of the precipitation lines.

†Hemagglutination titers expressed as the tube numbers of serial double dilutions with 1:10 in Tube No. 1.

There is some evidence that heating reduces the allergenicity of cow's milk. Two of Heiner and associates'[17] 7 patients improved when evaporated milk replaced fresh milk. All of our patients reacted to milk reconstituted from dried milk powder, although we confirmed the observation of others[18] that spray-dried milk preparations, modified and unmodified, are slightly less antigenic than fresh whole milk. Two of 3 of our infants who were tested with Allergilac also reacted violently. The third infant (Patient 6) was sensitive only to bovine serum albumin. He tolerated Allergilac, although antigenic bovine serum albumin was present. The reduced antigenicity of spray-dried milk has also been shown by using the P-K (Prausnitz-Küstner) or PCA (passive cutaneous anaphylaxis) reaction as a measure of antigenicity. Similac, though less antigenic, has been reported to react with antiserum to casein, alpha lactalbumin, and beta lactoglobulin. Immunoglobulins and serum albumin of cow's milk are relatively heat labile.[19, 20] Even Nutramigen, which is a hydrolysate, retained the antigenic properties of all the milk proteins which we tested. However, Nutramigen was tolerated well by an infant who developed a severe reaction to 5 ml. of fresh milk.

In 4 cases, ingestion of cow's milk during the first days of life was not detrimental, suggesting that a period of sensitization preceded the onset of symptoms. Infants with chronic diarrhea due to milk allergy frequently do not improve immediately upon replacement of cow's milk by milk substitutes. When milk sensitivity is suspected in such cases therefore, milk must be withheld for at least 3 weeks before the effects can be assessed.

Allergy to milk proteins is usually of limited duration. Most patients recover by the age of one year.[10] Two of our patients have recovered; only one of the others has reached the age of one year.

Serologic tests of antibodies to cow's milk are usually of limited value in the diagnosis of milk allergy,[21, 22] the notable exception being the strongly positive precipitation reactions found in milk-induced chronic respiratory disturbances, and in milk-induced anemia.[17, 23] It is of interest therefore that 3 of our patients had strongly positive precipitating and hemagglutinating reactions. No positive precipitin reactions occurred in our 54 control subjects. A similar phenomenon in

milk-induced diarrhea was observed by Silver and Douglas.[24]

The reason why only 3 patients had high antibody titers to cow's milk may be that these 3 patients were given regular feedings of cow's milk during the first month of life, whereas the others were given either none or infrequent feedings during the first month. We have shown that even normal infants who are fed cow's milk during the first month of life attain higher titers of milk antibodies than infants who are initially breast fed.[30]

The intestinal histology of patients with milk sensitivity has been found to be normal by some and abnormal by others.[25, 26] Both of our patients had biopsy specimens taken some weeks after cow's milk was discontinued. In Patient 2 there were definite pathological findings, though not as severe as in celiac disease.

Antibody titers to isolated milk proteins were not limited to the proteins producing clinical reactions. This is further evidence that the antibodies detected by these tests may not be involved in the clinical allergic reaction.

In many ways milk sensitivity resembles gluten-dependent enteropathy. In both instances the first ingestion is often harmless. Steatorrhea may occur after ingestion of gluten or beta lactoglobulin, though it seems to be more common in gluten sensitivity.[27, 28] Sometimes milk sensitivity is followed by gluten-induced enteropathy.[29, 31] It may therefore be of significance that gluten, like beta lactoglobulin, has a high content of glutamine.

SUMMARY

Six cases of milk allergy manifested by diarrhea and vomiting have been described. Five of these were induced by beta lactoglobulin, one by bovine serum albumin. Abnormally high levels of hemagglutinating and precipitating milk antibodies were found in 3 patients. Steatorrhea was found in none of the 4 cases in which it was sought. Intestinal biopsy was normal in one case and abnormal in another. Soybean formula was an adequate substitute in all cases.

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